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Technical note

Determination of the postmortem interval by Laser Induced Breakdown Spectroscopy using swine skeletal muscles

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ABSTRACT

Skin and muscle samples are useful to discriminate individuals as well as their postmortem interval (PMI) in crime scenes and natural or caused disasters. In this study, a simple and fast method based on Laser Induced Breakdown Spectroscopy (LIBS) has been developed to estimate PMI using swine skeletal muscle samples. Environmental conditions (moisture, temperature, fauna, etc.) having strong influence on the PMI determination were considered. Time-dependent changes in the emission intensity ratio for Mg, Na, Hα and K were observed, as a result of the variations in their concentration due to chemical reactions in tissues and were correlated with PMI. This relationship, which has not been reported previously in the forensic literature, offers a simple and potentially valuable means of estimating the PMI.

1. Introduction

The postmortem interval (PMI) refers to the time since death and its estimation is extremely important in criminal, civil and forensic investigations [1]. Postmortem decomposition is reported to begin immediately after death involving the breakdown of soft tissues due to the autolysis and putrefaction processes [2], and often with superimposed insect activity [3]. A death event is followed by a number of complicated chemical reactions which continue to occur within the cells, and cause specific time-dependent changes in metabolism and sub-cellular structures, enabling to determine PMI [1]. Many methods have been employed to accurately determine the PMI, also known as time of death (TOD), but the accuracy of these methods still leaves a high time uncertainty and needs improvements [4]. From an entomological point of view, since insect growth and development are heavily influenced by geographical, topological and environmental conditions, the difficulty in accounting for the conditions at the scene prior to discovery introduces some uncertainty in application. This variability in the environmental conditions made a well-characterized foundation of workable information difficult to attain [5]. Actually, methods of PMI estimation include body temperature measurements and postmortem chemical changes in the body [4]. In addition, some methods have also focused on the degradation of nucleic acids including DNA and RNA [1,6–8] and proteins within different tissues [9,10]. However, these methods require specialized staff and laboratories, and in some cases complicated and costly analysis procedures.

A significant relationship has been established between the ratio of postmortem sodium and potassium concentration variation with PMI in wistar rat serum [11] and human blood [12] samples. However, authors showed that internal and external environmental factors as well as the cause of death significantly affect PMI prediction due to the fast changes of this ratio in the serum and plasma samples (0–12 h) [13]. On the other hand, individual biological variability also limits the usefulness of PMI prediction based on these ratio measurements. Ideally, the concentration of the constituents at any given postmortem interval should display minimal individual biological variation and should be independent of environmental conditions.

Laser Induced Breakdown Spectroscopy (LIBS) has been a subject of research for the past few decades because of its unique features and wide variety of applications in various fields [14,15]. In recent years, LIBS has become a powerful analytical tool because of its ability to carry out a rapid qualitative and quantitative analysis of different samples [16,17]. LIBS analyzes a sample by direct measurement of the atomic emission of the elements from laser-induced plasma generated by the ablation of the sample, providing an immediate spectral fingerprint which is representative of its elemental composition [18]. Moreover, the technique requires little or no sample preparation as opposed to biochemical methods.

The spatial distribution of volumetric energy density generated by laser irradiation drives all pulsed laser ablation processes [19]. In case of laser–tissue interaction, this distribution is controlled by the incident radiant exposure and the optical absorption and scattering properties of the tissue. A detailed description of laser–tissue interaction has been the subject of many reviews discussing general aspects of this process [20–22]. Specific laser ablation processes resulting from nonlinear absorption have also been considered [23,24].

This paper focuses on the development of a simple, fast and direct analytical method for the determination of PMI based on LIBS using muscle tissue samples. Swine muscle tissues were selected due to similar

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physiological decomposition with that of human muscle tissues. Different environmental conditions (moisture, temperature, fauna, etc.) having strong influence on the PMI determination have been considered.

2. Materials and methods

2.1. Sample preparation

The study was conducted with the skeletal muscle samples of two pigs obtained from a licensed industrial slaughterhouse with all necessary certificates in compliance with the formal requirements of the European Union. The pigs were not killed only for the purpose of carrying out this experiment rather they were randomly selected from the stock killed for daily consumption purpose. Both pigs were brought from similar feeding and growing conditions. Duplicate samples of skeletal muscle (500–600 g) from each pig were stored in three different experimental conditions, i.e., a closed plastic container, in open air, and in open air covered with a metal mesh, keeping in the same place at ambient temperature and protected from rain. From each sample three sub-samples were measured at 0, 1, 2, 3, 7, 14, 21, 28 and 35 days, where zero refers to the day the pigs were killed. To ensure sample homogeneity, tissues were lyophilized. 250 mg of tissue was ground using an agate mortar and pestle for 5 min and subsequently, compressed into a compact pellet using a hydraulic press at 10 t/cm^2. The thickness and diameter of the pellets were around 2 mm and 12 mm, respectively. The pellets were directly analyzed without further preparation.

2.2. LIBS set-up

The LIBS set-up and methodology used in the present work have already been described elsewhere [18]. Thus, we only describe the experimental conditions relevant to the study presented here. Fig. 1 shows a schematic view of the experimental setup. LIBS measurements were obtained using a Q-switched Nd:YAG laser (Quantel, Brio model) operating at 1064 nm, with a pulse duration of 4 ns full width at half maximum (FWHM), 4 mm beam diameter and 0.6 mrad divergence. Samples were placed over an X–Y–Z manual micro-metric positionator with a 0.5 μm stage of travel at every coordinate to ensure that each laser pulse impinged on the pellet. The laser beam was focused on the surface of the pellet with a 100 mm focal-distance lens, producing a spot of 100 μm in diameter measured with a microscopic-micrometer. The laser fluence was fixed to 20 J/cm^2 and the repetition rate was 1 Hz. Emission from the plasma was collected with a 4-mm aperture and a 7 mm focus fused silica collimator placed at 3 cm from the sample, and then focused into an optical fiber (1000 μm core diameter, 0.22 numerical aperture), coupled to a spectrometer. The spectrometer system was a user-configured miniature single-fiber system EPP2000 StellarNet (Tampa, FL, U.S.A.) with a charged coupled device detector (CCD). A grating of 300 l/mm was selected which allowed to achieve a spectral resolution of 0.5 nm using a 7 μm entrance slit. The wavelength range used was from 200 to 1000 nm. Therefore, 2048 data points were recorded for each sample. The detector integration time was set to 1 ms. To prevent the detection of bremsstrahlung, the detector was triggered with a 5 μs delay time between the laser pulse and the acquired plasma radiation using a digital delay generator (Stanford model DG535).

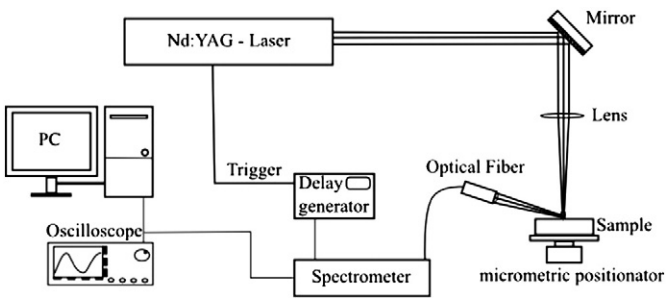

Fig. 1. The experimental setup, including a Nd:YAG laser, a delay generator, a micrometric positionator, a 1 m optical fiber, and an optical charge coupled device (CCD) spectrometer.

2.3. LIBS measurements

All the pellets were analyzed at room conditions. Five LIBS spectra were obtained averaging 20 individual spectra for each pellet, that were measured by impinging the laser beam in a fresh position on the surface along a vertical straight line avoiding the areas altered by the previous shots moving the pellet about 1 mm with each laser pulse. The total acquisition time was less than 2 min taking into account the spectrometer integration time and the laser pulse repetition.

2.4. Data analysis

Under local thermodynamic equilibrium (LTE) the intensity of an atomic emission spectral line can be described as [16]:

$$I = \frac{hcg_k A_{ki} N}{4\pi\lambda Z} \exp\left(-\frac{E_k}{kT}\right) \quad (1)$$

where h is the Planck's constant, c the velocity of light, g_k the degeneracy of a given energy state, A_{ki} the transition probability (Einstein A coefficient), N the total specie population, λ the emission wavelength, Z the partition function, usually taken as the statistical weight of the ground state, E_k the energy of the upper state, k the Boltzmann's constant and T the plasma temperature.

For a specific spectral line, all the parameters are known except T and N. Therefore, the emission intensity (I) is only related with T and N. If no significant variation of T is assumed along the experimental measurements, a proportional relationship between I and N can be established [17]. However, it is important to note that as LIBS experiment progresses, although all the experimental parameters (integration time, laser power, etc.) remain constant, plasma temperature could vary due to factors such as the matrix-dependence ionization potential of the elements. Therefore, plasma temperature depends on sample composition and specie population [25–27]. From the instrumental point of view, plasma temperature can be fixed to a constant value by controlling the energy of laser pulses by adjusting the working voltage. One way to check plasma temperature is by real time monitoring of the ratio of two atomic

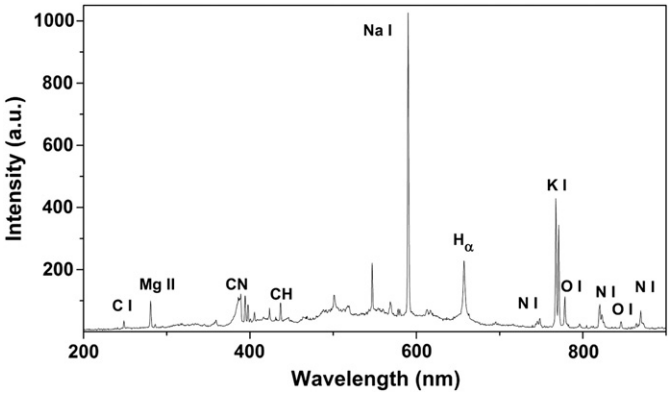

Fig. 2. Typical LIBS spectrum of a lyophilized and pellet-compacted swine skeletal muscle sample stored in air.